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Quantitative analysis of renal medullary anatomy in rats and rabbits

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Quantitative analysis of renal medullary anatomy in rats and rabbits. The mean renal tubular diameters, number of tubules per unit of cross-sectional area, and fraction of the total volume occupied by each medullary structure were determined at various levels of the renal medulla of the rat and rabbit. Statistical estimates of anatomic variables were made using spatial sampling techniques on histologic sections. Osmotic diuresis and renal venous occlusion were used to allow fixation of renal tubules and blood vessels in an open state. The distribution of volume fractions of medullary structures are similar in rats and rabbits. Diameters of outer medullary tubular segments and inner medullary thin limbs of Henle are also similar in rats and rabbits. Marked differences between rats and rabbits, however, are seen in the size and number of collecting ducts in the inner medulla. Rabbit inner medullary collecting ducts increase in diameter and decrease in number in the papillary direction relatively closer to the cortex than do those of the rat. Luminal diameters of papillary collecting ducts are more than twice as great in the rabbit as in the rat. An additional finding was that short loops of Henle in the rabbit have their bends relatively closer to the cortex than those of the rat. The quantitative anatomic data derived in this study, when combined through mathematical modeling with knowledge of transport properties of renal tubular membranes, should lead to a clearer understanding of renal function.

Analyse quantitative de l'anatomie de la médullaire rénale chez le rat et le lapin. Les diamètres tubulaires moyens, le nombre de tubules par unité de surface de la coupe et la fraction du volume total occupée par chaque structure médullaire ont été déterminés à différents niveaux de la médullaire du rat et du lapin. Les estimations statistiques des variables anatomiques ont été faites au moyen des techniques d'échantillonnage dans l'espace sur des coupes histologiques. La diurèse osmotique et l'occlusion de la veine rénale ont été utilisées pour permettre la fixation des tubules et des vaisseaux en situation de réplétion. La distribution des fractions du volume appartenant à chacune des structures médullaires est semblable chez le rat et le lapin. Les diamètres des segments tubulaires de la médullaire externe et des anses grêles de Henle de la médullaire interne sont semblables, eux aussi, chez le rat et le lapin. Des différences importantes, cependant, sont observées dans la taille et le nombre des canaux collecteurs de la médullaire interne. Les canaux collecteurs de la médullaire interne du lapin augmentent en diamètre et diminuent en nombre en direction de la papille plus près du cortex, comparativement, que chez le rat. Les diamètres luminaux des canaux collecteurs papillaires sont deux fois plus grands chez le lapin que chez le rat. Une constatation supplémentaire est que les anses de Henle courtes du lapin ont leur extrémité plus près du cortex, comparativement, que chez le rat. Les résultats anatomiques quantitatifs obtenus dans ce travail devraient conduire à une meilleure compréhension du fonctionnement rénal quand ils seront combinés, dans les modèles mathématiques, aux données des propriétés de transport des membranes tubulaires rénales.

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A great deal of effort has been expended in recent years in the measurement of the transport properties of renal tubular membranes [for recent reviews, see References 1–3]. Most of these measurements have been made in the rat and the rabbit. Such investigations have resulted in considerable advances in the understanding of the mechanism of urine formation by the kidney. Transport of solutes and water by the nephron, however, cannot be completely characterized without knowledge of the number and dimensions of renal tubules at various levels of the kidney as well as the volume of the interstitial and vascular spaces.

Quantitative anatomical data describing the mammalian kidney is presently available from a number of sources [e.g., 4–9]. These data, however, were obtained from many different species, using several methods. It is desirable to have comprehensive data describing the renal anatomy of individual species measured under uniform conditions with a single technique. This study provides such data for the renal medulla of the rat and the rabbit, the two species in which most measurements of nephron transport properties have been made. Variations along the cortico-medullary axis in the mean diameters of each tubular segment, the number of tubules per unit of cross-sectional area, and the fraction of the total medullary volume occupied by each structure are presented.

Methods

Four adult male Sprague-Dawley rats and two adult female New Zealand rabbits were used in these

studies. Animal weights are reported in Table 3. The technique of mannitol diuresis and renal venous occlusion used by Kriz [10] was employed to fix tubules and blood vessels in an open state.

Animals were deprived of food, but not water, overnight prior to sacrifice. Rats were anesthetized with Inactin (Promonta, 16 mg/100 g of body wt, i.p.). Rabbits were anesthetized with ether. A cannula was placed in the jugular vein in rats and in the femoral vein in rabbits, and 2 ml/100 g body wt of 15% mannitol in 0.45% sodium chloride was given i.v. over a period of 10 to 15 min. A midline abdominal incision was made and the left kidney exposed. The left kidney was freed by blunt dissection. The renal vein was ligated within 5 min of the completion of the mannitol injection, during which time a brisk osmotic diuresis had ensued as evidenced by rapid distention of the bladder. Renal venous occlusion was maintained for 75 to 120 sec prior to resection of the kidney with the ligatures intact. The kidney was placed immediately into 10% formalin pre-chilled to 5°C. The contralateral (right) kidney was then resected and weighed. The cortex of the contralateral kidney was dissected from the medulla with a sharp scalpel, exposing the surface at the boundary of the cortex and medulla (cortico-medullary surface). Imprints of the entire cortico-medullary surface were made by coating the surface with a dilute solution of Wool Green S stain (Allied Chemical) and rolling the surface over a sheet of plain white paper. The cortico-medullary surface area was then estimated from the imprints by planimetry.

The left kidney was removed from the fixative after two days. Excess renal tissue was trimmed from the specimen leaving the papilla and a block of inner medulla, outer medulla, and cortex along the longitudinal axis defined by the papilla. The specimen was embedded in paraffin, and consecutive 5-μ sections were made from the cortico-medullary junction to the papillary tip perpendicular to the cortico-papillary axis. Representative sections from various positions throughout the medulla were stained with hematoxylin and eosin for observation.

In one rat (R-5), renal venous occlusion was not used, and the kidney was fixed with Carnoy's solution [8]. The kidney was trimmed, as previously described, immediately after removal from the animal. With this technique the tubules were not well dilated. Furthermore, it was not possible to differentiate thin limbs of Henle from vasa recta because of the absence of red blood cells in the latter. Consequently, only a partial analysis was done on this animal.

Analysis of the tissue specimens employed a trinocular light microscope (American Optical, model XL10TG-QW) fitted with a projecting prism. The microscopic image was projected onto large sheets of white paper at a magnification of approximately ×525. Recalibration was done throughout the analysis using a stage micrometer. All microscopic sections were examined by one observer (M.A.K.).

Volume fraction analysis. A two-dimensional systematic point count technique [11, 12] was used in this study to determine the volume fraction of structures at various levels of the renal medulla. With this technique, the fraction of points in a regular array falling on a given type of structure gives an unbiased estimate of the volume fraction of that type of structure, i.e., the fraction of the total volume occupied by the structure. A similar technique was used by Munkacsi and Palkovits [6] to determine the volume fraction of thin limbs in several species. A square grid of one hundred points was used with each point separated from its nearest neighbor by 20 mm (38 μ relative to the specimen). The number of points falling on each of the following structures was counted: collecting duct cells (CDC), collecting duct lumens (CDL), thin limbs of Henle (TL), thick ascending limb cells (TALC), thick ascending limb lumens (TALL), cells of the straight portion of the proximal tubule (PTC), lumens of the straight portion of the proximal tubule (PTL), interstitium (I), and vasculature (V). In general, it was not difficult to differentiate among various types of tubules using criteria discussed by Trump and Bulger [13]. No attempt was made to determine the volume fraction of thin limb cells since the thickness of the cells was about the same as the size of the points used. Therefore, the volume fraction of thin limbs is assumed to include both cells and lumens. "Interstitium" is defined here as any space not occupied by either renal tubules or vasculature. "Vasculature" includes both vasa recta and capillaries and is identified by the presence of red blood cells within a lumen. As with the thin limbs, the vasculature as defined here includes both endothelial cells and lumens. When a point fell on a boundary between two structures, one half was counted for each structure. For this analysis, five fields of one hundred points each were counted at each medullary level. Microscopic fields were chosen for analysis near the center of each tissue section in regions where tubules appeared round in cross-section, indicating a perpendicular cut.

Number density analysis. The number densities of each tubule type, i.e., the number of structures occurring per unit of area, was determined for each microscopic field observed in the volume fraction

analysis. The total number of structures falling within a square 180 mm on a side (0.343 mm on a side, relative to the specimen) was counted. Tubules whose lumens fell on the left or upper borders of the square were included in the count; those falling on the right or lower borders were ignored. The mean number density among the five fields counted is reported for each tissue section studied.

Calculation of mean diameters. The mean inner and outer diameter of each type of tubule can be calculated from the volume fraction of the lumens (f_L) and cells (f_C) and number density (ρ), assuming each tubule is circular in cross-section. The mean inner diameter (D_I) is given by

$$D_I = 2\sqrt{f_L/\pi\rho} \quad (1)$$

and the outer diameter (D_O) by

$$D_O = 2\sqrt{(f_L + f_C)/\pi\rho}. \quad (2)$$

Variability estimates. The standard deviation (S^i) of a volume fraction determination, using the systematic point count technique, is estimated from the total number of points counted (N) and the volume fraction (f_i) of structure i [11, 12] and is given by

$$S_i = \sqrt{f_i/N}. \quad (3)$$

Additional estimates of variability were made by repeating the analysis of volume fractions, number densities, and mean diameters on eight sections from rat R-4 (four sections each from the outer and inner medulla). Repeat determinations were done at a time remote from initial determinations without recollection of prior results.

In Table 1, standard deviations for volume fraction determinations estimated from repeat measurements are compared with standard deviations predicted by equation 3. In general, measured standard deviations were only slightly greater than the predicted values. Excess deviation above the theoretical is due primarily to moderate inhomogeneity among the fields analyzed, a factor not considered in the derivation of equation 3.

Table 1. Standard deviations (SD) for volume fraction determinations (f_i)[a]

Structure	Location	f_i (N)[b]	Measured SD[c]	Calculated SD[d]
CDL	OM	0.060(4)	0.018	0.011
CDL	IM	0.178(4)	0.024	0.019
CDC	OM	0.044(4)	0.019	0.009
CDC	IM	0.207(4)	0.016	0.020
TALL	OM	0.119(4)	0.025	0.015
TALC	OM	0.170(4)	0.023	0.018
TL	IM	0.334(4)	0.029	0.026
V	OM,IM	0.211(8)	0.053	0.020
I	OM,IM	0.093(8)	0.043	0.013

[a] Abbreviations: CDL, collecting duct lumens; CDC, collecting duct cells; TALL, thick ascending limb lumens; TALC, thick ascending limb cells; TL, thin limbs; V, vasculature; I, interstitium; OM, outer medulla; IM, inner medulla.
[b] Mean volume fraction, f_i, for N initial determinations.
[c] SD estimated from N repeat determinations.
[d] SD predicted by equation 3 (see Methods section).

Table 2 shows measured standard deviations for number density determinations estimated from repeat measurements. The values presented are standard deviations of the difference between the initial and repeated measurements, divided by the initial measurement. The standard deviations were less than six percent of the number density for structures with number densities greater than 200 mm^{-2}. Since mean tubular diameters are calculated from the volume fraction and number density data, errors tend to parallel those seen in Tables 1 and 2.

Table 2. Standard deviations (SD) for number density determinations (ρ)[a]

Structure	Location	ρ (N)[b]	Measured relative SD[c]
CD	OM	92(4)	0.184
CD	IM	429(4)	0.040
TAL	OM	447(4)	0.058
TL	IM	1156(4)	0.048

[a] Abbreviations: CD, collecting ducts; TAL, thick ascending limb; TL, thin ascending limb; OM, outer medulla; IM, inner medulla.
[b] Mean number density, ρ (mm^{-2}), for N initial determinations.
[c] Relative SD estimated from N repeat determinations (SD of difference between final and initial measurements divided by initial measurement).

Results

Gross features of animals studied. Table 3 shows the animal and kidney weights, and characteristic macroscopic renal dimensions of the animals studied. The boundary between the inner and outer stripes is not well defined in the rabbit. Consequently, the thickness of the inner stripe is measured from the approximate midpoint of the zone in which straight segments of proximal tubules become thin descending limbs. This zone is about 500 microns wide.

Since the characteristic renal dimensions differ among the animals studied, subsequent data in this paper are plotted against lengths which are normalized by dividing by a convenient characteristic length, *viz.*, the inner medullary axial length (Table 3). Thus, in terms of normalized distances, the junction between the inner and outer medulla lies exactly one unit from the papillary tip in all animals. In rats,

Table 3. Gross characteristics of animals studied

Species, animal no.	Animal wt, *g*	Contralateral kidney wt, *g*	Inner medullary axial length[a] *mm*	Outer medullary thickness *mm*	Inner stripe thickness *mm*	Cortico-medullary surface area *mm²*
Rat:						
R-2	235	—[b]	4.5	—[b]	—[b]	—[b]
R-3	271	1.37	4.8	2.0	1.5	200
R-4	220	1.34	5.5	2.2	1.5	213
R-5[c]	370	2.21	4.0	2.0	1.5	361
Rabbit:						
H-1	1590	8.55	6.0	—[b]	1.6	756
H-2	1680	8.54	5.5	2.7	2.1	855

[a] Distance from papillary tip to outer–inner medullary junction.
[b] Measurement not made.
[c] No renal venous occlusion; fixed in Carnoy's solution.

the cortico-medullary junction lies approximately 1.45 normalized units from the papillary tip, and the boundary between the inner and outer stripes lies approximately 1.30 normalized units from the papillary tip. The corresponding normalized distances in rabbits are approximately 1.50 and 1.30, respectively.

Volume fractions. Figure 1 shows mean volume fractions of medullary structures plotted against the normalized distance from the papillary tip in rats. The fraction of the outer stripe volume not accounted for in Figure 1 is taken up by straight segments of proximal tubules. The following observations can be made: *a)* The interstitium (I) makes up an increasing fraction of the medullary substance from the outer medulla to the papillary tip. *b)* Consistent with previous studies [14–16], vascular structures (V) make up a much greater proportion of the outer medulla than of the inner medulla. *c)* Thin limbs (TL) of Henle compose a large fraction of the inner medulla, particularly the outer portion. *d)* Collecting ducts are most dominant in the inner medulla with an increasing volume fraction toward the papillary tip. This increase is due to an increase in the volume fraction of collecting duct cells (CDC), the volume fraction of lumens (CDL) remaining nearly constant throughout the inner medulla. *e)* Thick limbs of Henle make up a greater proportion of the inner stripe of the outer medulla than of the outer stripe. *f)* Thick ascending limb cells (TALC) take up a greater fraction of outer medullary volume than do the lumens (TALL).

Figure 2 shows volume fractions for rabbits plotted in the same manner as Figure 1. Volume fractions in rabbits are similar to those in rats with the following exceptions: *a)* the interstitium is more prominent in the outer medulla and outer portion of the inner medulla of the rabbit; *b)* vascular structures take up

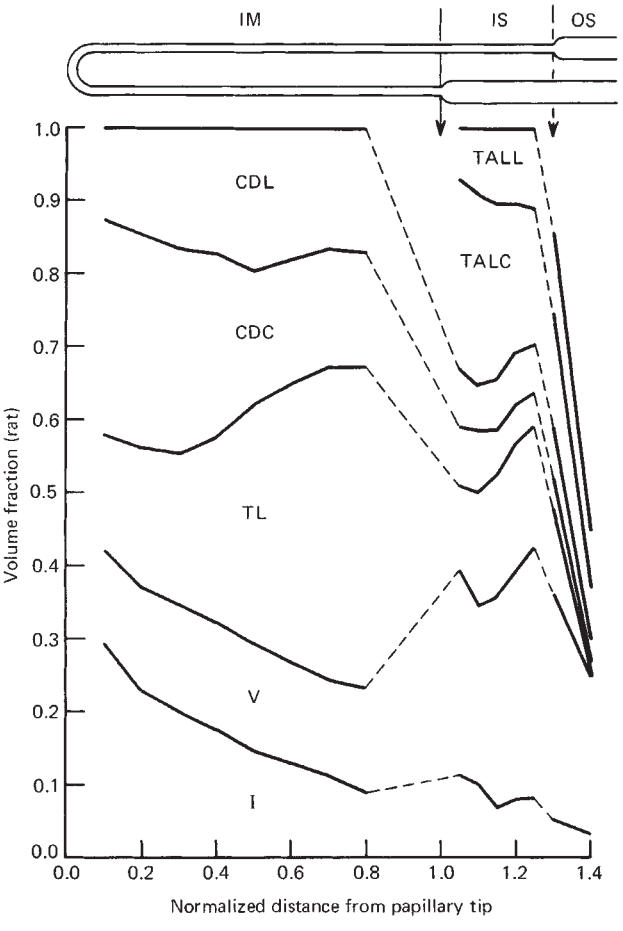

Fig. 1. *Volume fraction distributions of structures which compose the rat renal medulla.* Volume fraction of a given structure is given by the vertical distance between adjacent lines. Values plotted are means of all data from three rats (R-2, R-3, R-4). Schematic diagram of long loop of Henle demonstrates location of inner medullary zone (IM), inner stripe (IS), and outer stripe (OS) of outer medulla. Other terminology is defined in text.

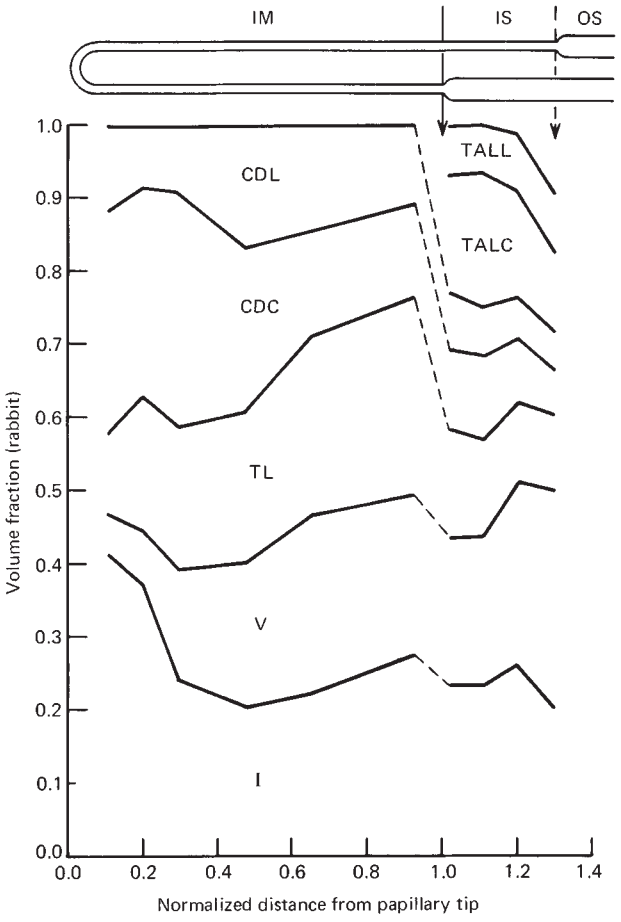

Fig. 2. *Volume fraction distributions of structures which compose the rabbit renal medulla.* Volume fraction of a given structure is given by the vertical distance between adjacent lines. (Terminology is the same as in Fig. 1.)

a relatively greater proportion of the outer portion of the inner medulla in rabbits; and *c)* thin limbs of Henle take up a relatively smaller proportion of the outer portion of the inner medulla in rabbits. Volume fraction data in the outer stripe of the outer medulla were obtained in only one animal (H-2) and so is not plotted in Figure 2.

Number densities. The distributions of number densities of collecting ducts and ascending limbs in the rat renal medulla are shown in Figures 3 and 4, respectively. Each point represents results from the examination of one tissue section. The number density of collecting ducts in the outer medulla increases from the cortico-medullary junction to the inner-outer medullary junction. The number density of collecting ducts is nearly constant through most of the inner medulla, but tapers off rapidly close to the papillary tip. In the outer medulla, the number density of thick ascending limbs also increases from the cortico-medullary junction to the inner–outer medullary junction. The number density of ascending thin limbs in the inner medulla is obtained by dividing the total number of thin limbs by two. Ascending thin limbs are quite dense in the outer portion of the inner medulla, but decrease toward the papillary tip.

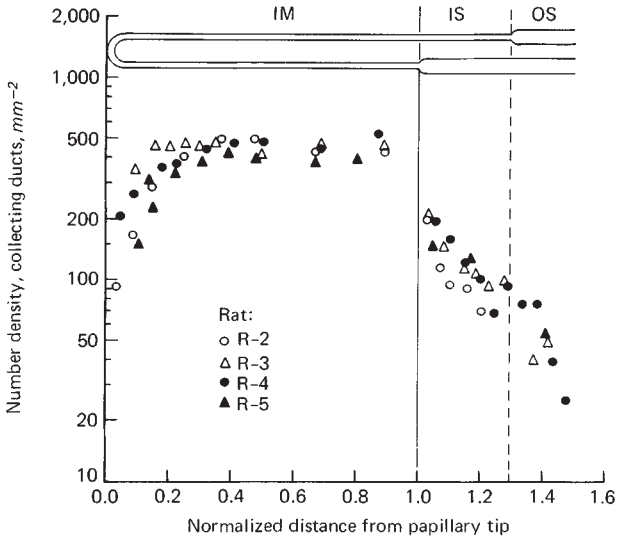

Fig. 3. *Number density distribution of collecting ducts in rat renal medulla.*

The distributions of number densities of collecting ducts and ascending limbs in the rabbit renal medulla are shown in Figures 5 and 6, respectively. The number densities of collecting ducts in the outer medulla of the rabbit are distributed in a manner similar to those of the rat. Unlike the rat, however, the number density of collecting ducts in the inner

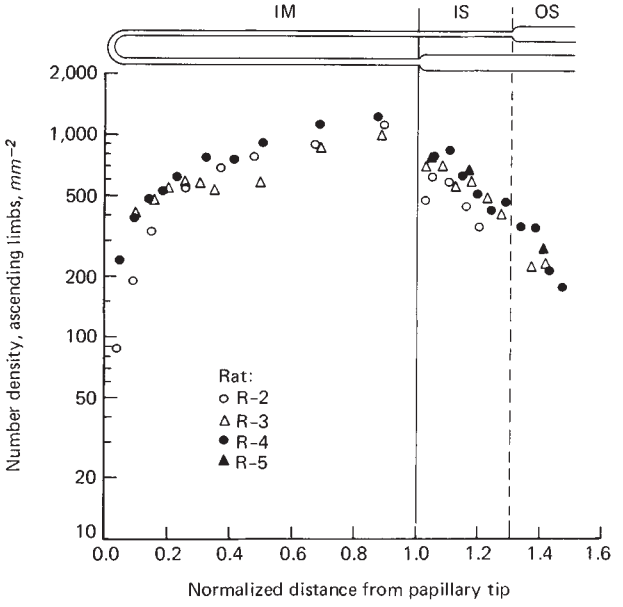

Fig. 4. *Number density distribution of ascending limbs in rat renal medulla.*

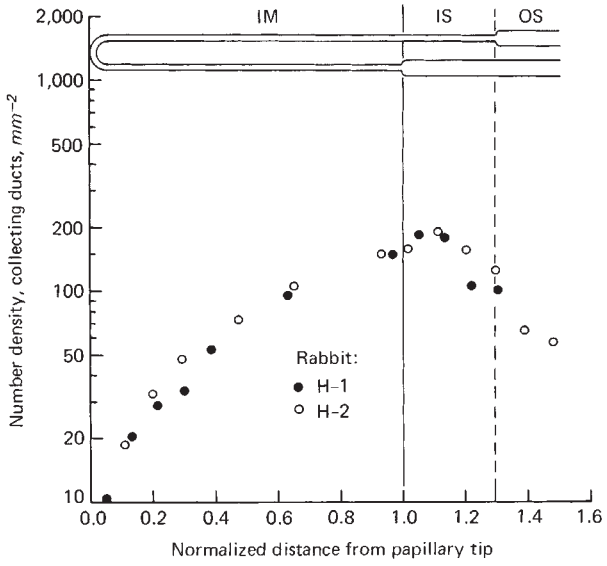

Fig. 5. *Number density distribution of collecting ducts in rabbit renal medulla.*

medulla of the rabbit decreases continuously from the inner–outer medullar junction to the papillary tip. The distribution of number densities of ascending limbs in the rabbit qualitatively resembles that of the rat. The number densities of ascending limbs in the rabbit, however, tend to be lower than those of the rat, except near the cortico-medullary junction.

Ascending limb:Collecting duct ratios. A consideration of the ratio of the number of ascending limbs (AL) to the number of collecting ducts (CD) throughout the length of the medulla leads to some important contrasts between the rat and the rabbit. In the rat

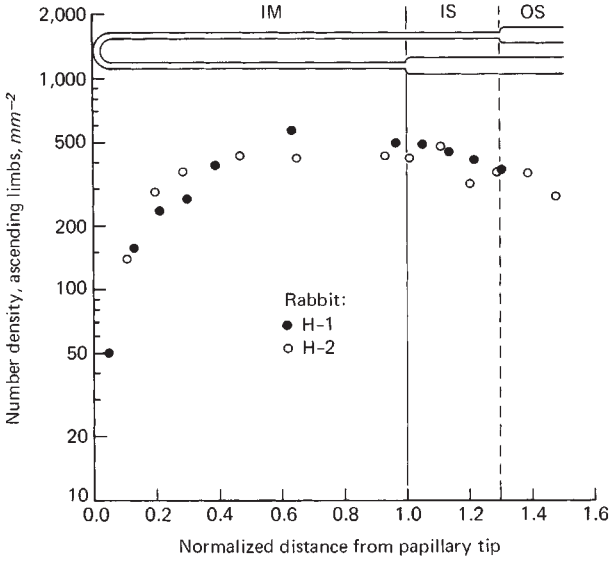

Fig. 6. *Number density distribution of ascending limbs in rabbit renal medulla.*

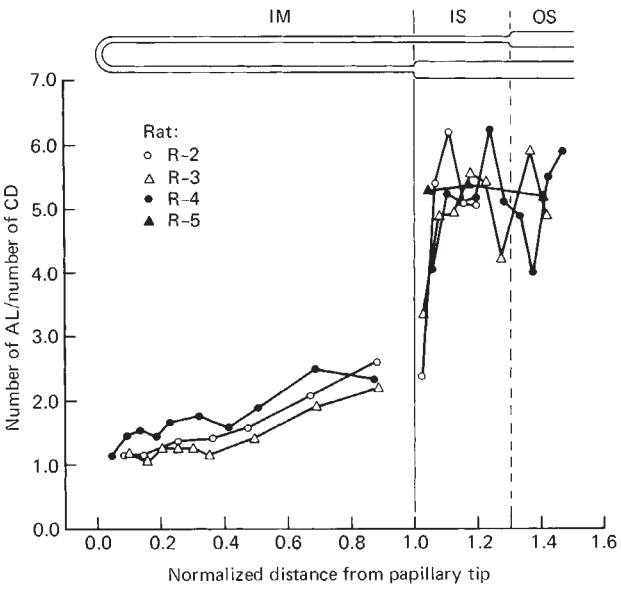

Fig. 7. *Ratio of ascending limbs (AL) to collecting ducts (CD) in rat renal medulla.*

(Fig. 7), the AL:CD ratio remains in the range 4.0 to 6.3 throughout the outer medulla, except near the inner-outer medullary border where the ratio rapidly decreases toward 2.4. As the papillary tip is approached in the inner medulla, the AL:CD ratio gradually decreases to about 1.0. In the rabbit (Fig. 8), the AL:CD ratio is about 5 near the cortico-medullary junction, but falls to below 3 in the mid-outer medulla. Since the number of collecting ducts does not increase in the direction of the papilla [7], this AL:CD distribution indicates that many bends of Henle's loops occur in the mid–outer medulla of the rabbit. This contrasts with the rat in which most of the bends of short loops appear to occur very near

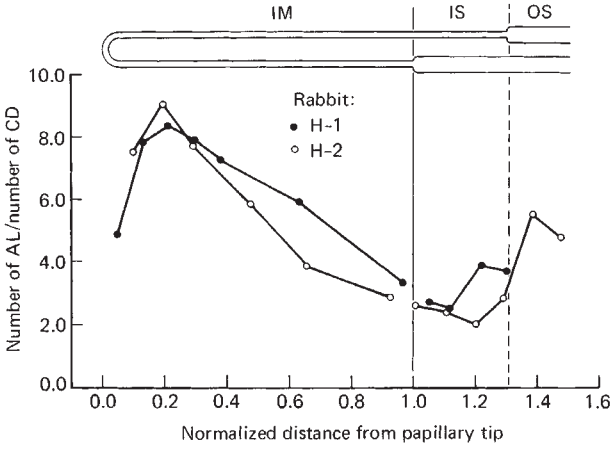

Fig. 8. *Ratio of ascending limbs (AL) to collecting ducts (CD) in rabbit renal medulla.*

the inner–outer medullary border. The distribution of AL:CD ratios in the inner medulla of the rabbit also differs markedly from that seen in the rat. The AL:CD ratio increases toward the papillary tip throughout most of the inner medulla of the rabbit, reaching a value of 8 to 9 at a normalized axial distance of 0.2 from the papillary tip. This contrasts with the steady decrease in the AL:CD ratio throughout the entire inner medulla of the rat (Fig. 7). A comparison of Figures 3 and 5 suggest that this difference results primarily from a sharper reduction in the number of collecting ducts closer to the inner-outer medullary border of the rabbit than occurs in the rat.

Tubule diameters. Figures 9–14 show the distributions of mean tubular diameters calculated from volume fractions and number densities using equations 1 and 2 (Methods section). Mean inner and outer diameters of collecting ducts in rats are shown in Figure 9. Because of the low number density of

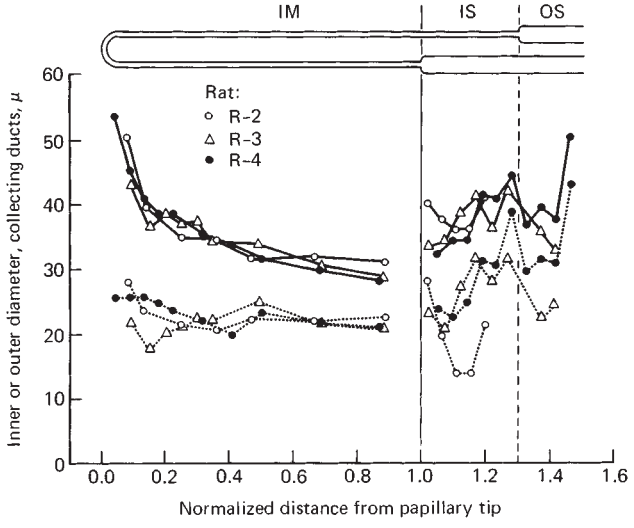

Fig. 9. *Mean inner and outer diameters of medullary collecting ducts in rats. Solid lines* indicate outer diameters; *dashed lines,* inner diameters.

collecting ducts in the outer medulla, there is considerable variation of mean diameters with distance. In rats R-3 and R-4, the cells become very thin and the luminal diameters become greater as the corticomedullary junction is approached in the outer medulla. In these two animals, the luminal diameters are greater in the outer medulla than in the inner medulla. In the third rat (R-2), the collecting duct cells of the outer medulla appeared swollen relative to those in rats R-3 and R-4, perhaps reflecting a failure of the technique to eliminate antidiuretic hormone effects in this rat [17]. In the inner medulla, the inner and outer diameters are much more consistent from rat to rat. The mean luminal diameter is surprisingly uniform throughout the length of the inner medulla, remaining between 20 and 28 μ. The thickness of collecting duct cells, however, increases throughout the length of the inner medulla. The mean outer diameter of collecting ducts increases from about 30 μ near the inner–outer medullary junction to greater than 50 μ near the papillary tip.

Mean collecting duct diameters in the renal medulla of the rabbit are shown in Figure 10. Mean diame-

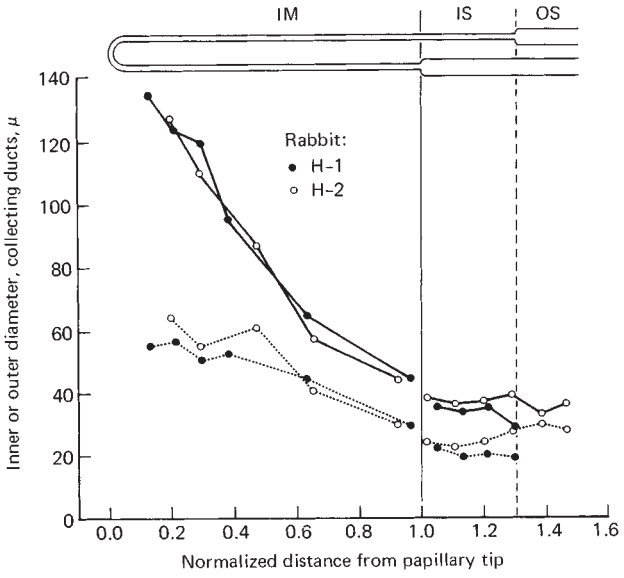

Fig. 10. *Mean inner and outer diameters of medullary collecting ducts in rabbits. Solid lines* indicate outer diameters; *dashed lines,* inner diameters.

ters of collecting ducts in the outer medulla are remarkably similar to those in the rat. In contrast to the rat, however, the luminal diameter and cell thickness of rabbit inner medullary collecting ducts increase markedly throughout the length of the inner medulla. The mean luminal diameter of collecting ducts increases from about 30 μ near the inner–outer medullary junction to about 60 μ in the papilla. The mean outer diameter increases from about 45 μ to greater than 130 μ in the papilla. Diameters of papillary collecting ducts increase even more than is indicated in Figure 10 in sections closer to the papillary tip, forming ducts of Bellini with a stratified epithelium resembling the lining of the outer surface of the papilla.

Outer diameters of thin limbs of Henle in the rat and rabbit are shown in Figures 11 and 12, respectively. As previously noted in the Methods section, it is not possible to estimate cell thicknesses in thin limbs because the height of cells is approximately the same as the size of the points used to estimate volume fractions. The qualitative distributions of thin

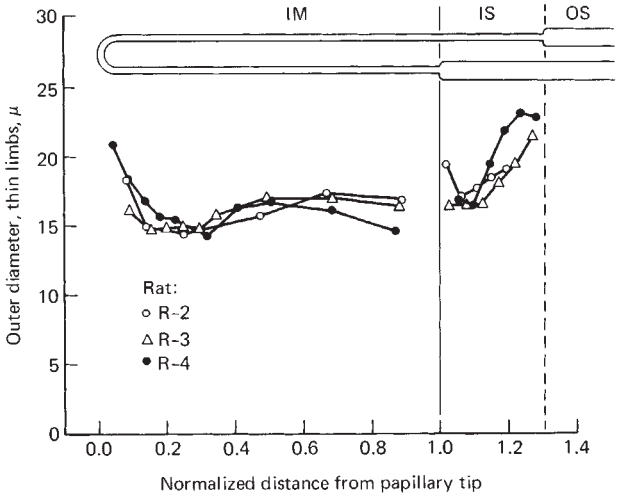

Fig. 11. *Mean outer diameters of thin limbs of Henle in rats.*

limb diameters are similar in the rat and rabbit. Thin limb diameters, however, are about 10 to 20% greater in the rabbit. In both species, the mean diameter of thin descending limbs in the outer medulla is greatest near the junction of the inner and outer stripes so as to form a smooth transition with the straight proximal tubules. (Mean diameters of straight proximal tubules near the zone of transition to thin descending limb: rat inner diameter, 18 μ; rat outer diameter, 37 μ; rabbit inner diameter, 22 μ; rabbit outer diameter, 37 μ.) Mean thin limb outer diameters appear to increase near the papillary tip. This observation is consistent with the results of Steinhausen [18] and Koepsell, Kriz, and Schnermann [9], demonstrating that the lumen of the thin descending limb becomes wider just before the bend of the loop. Since the diameters of thin ascending limb and thin descending limbs are not separately measurable by this method, it is not possible to directly compare these results with those of Koepsell, Kriz, and Schnermann [9] who measured diameters of individual thin ascending and descending limbs. It is of interest to note, however, that the mean thin limb diameters measured by us in the rat inner medulla (15 to 17 μ) are similar to or slightly greater than the mean between ascending limbs and descending limbs as measured by Koepsell et al [9]. These authors prepared their animals with a brisk osmotic diuresis, as done in this study, but achieved fixation of renal tissue by *in vivo* perfusion with glutaraldehyde. Thus, despite the fact that fixation by immersion of the entire kidney was used in the present study, there is no evidence of collapse of the thin limbs.

Figure 13 shows the mean inner and outer diameters of thick ascending limbs in the outer medulla of the rat. It is seen that the mean thickness of thick limb cells gradually decreases in the direction of the cortico-medullary junction. Allen and Tisher [19] have recently made similar observations and have demonstrated that the reduction of cell thickness correlates with a transition from a smooth to a rough-surfaced cell as the predominant cell type. Mean diameters of thick ascending limbs in the rabbit (Fig. 14) are similar to those of the rat. A reduction in cell thickness toward the cortico-medullary junction, however, is not clearly demonstrated in the rabbit.

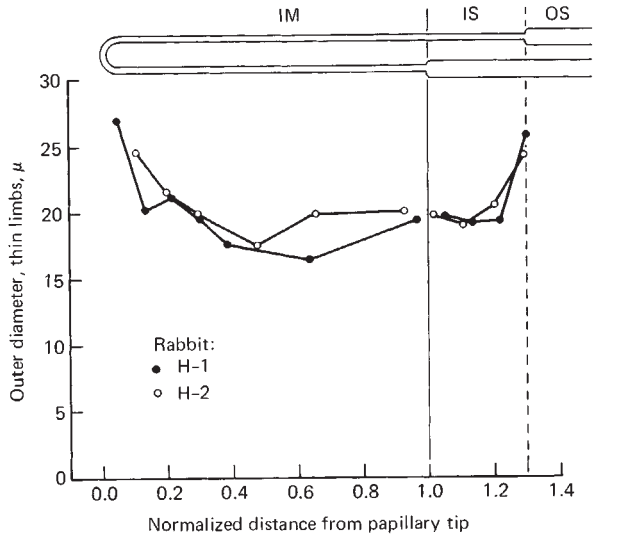

Fig. 12. *Mean outer diameters of thin limbs of Henle in rabbits.*

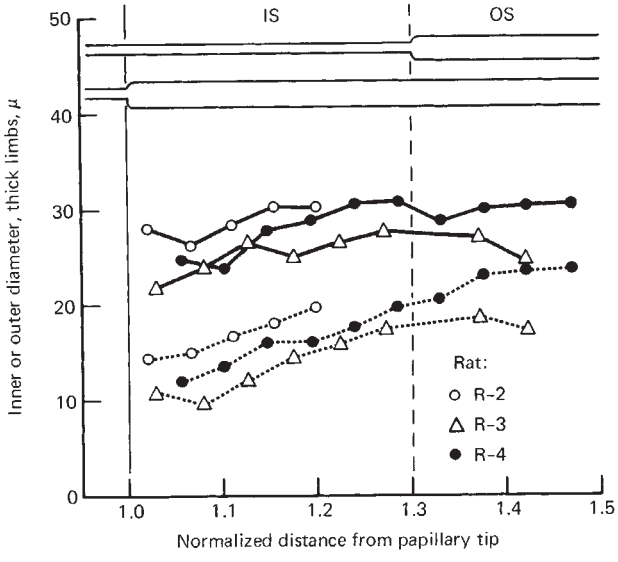

Fig. 13. *Mean inner and outer diameters of thick ascending limbs of Henle in rats. Solid lines* indicate outer diameters; *dashed lines,* inner diameters.

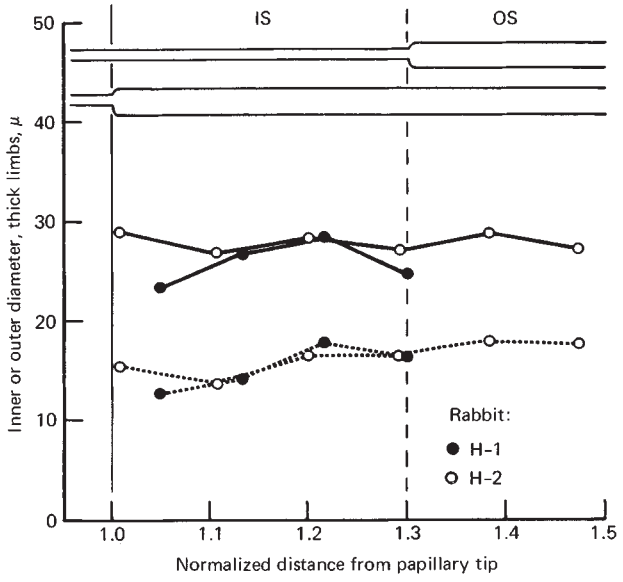

Fig. 14. *Mean inner and outer diameters of thick ascending limbs of Henle in rabbits. Solid lines* indicate outer diameters; *dashed lines,* inner diameters.

Discussion

The objective of this study has been to derive comprehensive data, measured under uniform conditions, describing the renal medullary anatomy of the rat and rabbit. To achieve this, we have used the method of Kriz [10], involving osmotic diuresis and renal venous occlusion, to fix tubules and vasculature in an open state. This method has yielded preparations of consistent quality. Thus, comparisons may be made between species or between levels of the medulla in a single species without concern that differences may have resulted from methodologic variations.

An important question that arises is how well the values obtained in this study describe the *in vivo* state. Clearly, the distribution of number densities should be affected very little. Measurements of tubule diameters and vascular volume, however, will be highly sensitive to experimental conditions. As is well recognized, *in vivo* tubule diameters are not static, but rather are markedly influenced by such factors as intraluminal pressure [20, 21] and antidiuretic hormone [17, 22, 23]. A similar statement must hold for vascular structures. Thus, tubular and vascular dimensions measured by a single histologic method cannot be totally descriptive of the dynamic *in vivo* state, regardless of the method used. The method used here tends to maximally distend tubules and vasculature, eliminating the variability which could occur if flow of fluid in the nephron and vasculature were not controlled. Consequently, the tubular diameters and vascular volumes measured here must probably be considered to represent upper bounds on the *in vivo* values.

Several quantitative anatomical differences between the rat and rabbit renal medullae may have functional significance. Differences in the collecting ducts of the two species are particularly noteworthy. As described by Sperber [4], a number of initial collecting tubules join in the cortex in various configurations according to the species involved to form a single cortical collecting duct. As noted by Oliver [7], these collecting ducts run downward into and through the outer medulla as long branchless segments. Many junctions between pairs of collecting ducts occur in the inner zone until only a few ducts of Bellini remain at the papillary tip. The findings of this study are consistent with this basic description. Striking differences, however, are seen between rat and rabbit in the size of inner medullary collecting ducts and in the distribution of collecting duct junctions. In the rat, the luminal diameter of the inner medullary collecting duct remains nearly constant throughout the length of the inner medulla, even up to the papillary tip (Fig. 9). Large ducts of Bellini are not seen. In contrast, the luminal diameter of the collecting duct in the inner medulla of the rabbit increases continuously toward the papillary tip (Fig. 10), finally forming extremely large ducts of Bellini with luminal diameters in excess of 100 μ. A marked contrast also exists between rats and rabbits in the distribution of collecting duct junctions. The number of collecting ducts decreases much more precipitously in the rabbit than in the rat as one progresses from the inner–outer medullary border to the papillary tip. These differences between rat and rabbit in the number and size of inner medullary collecting ducts could be responsible for considerable differences in the quantities of solute and water transported by the collecting ducts. Since the current theory of solute concentration in the inner medulla without active salt transport by the thin ascending limbs of Henle assumes an important role for the collecting ducts [24, 25], the anatomical dissimilarities between rat and rabbit collecting ducts may be functionally important. In addition, the structural differences demonstrated may be indicative of differences in the permeability or active transport capacity of the membranes.

An interesting observation is that the number densities of both ascending limbs and collecting ducts increase by a considerable degree in the outer medulla in the direction of the inner–outer medullary junction, particularly in the rat (Figs. 3–6). This is in seeming contradiction with the fact that the absolute

numbers of both structures decrease toward the inner medulla or, at most, remain unchanged as may be the case with collecting ducts in the outer medulla [7]. Since the number density is defined as the number of structures per unit of area, the total cross-sectional area of medullary substance perpendicular to the renal tubules (actually a three-dimensional curved surface [8]) must undergo a marked contraction in the direction of the inner medulla to account for the increasing number densities. Unfortunately, it is impossible to measure the areas of these surfaces. If the surfaces are thought of roughly as concentric hemispheres, however, it will be realized that their areas will be proportional to the square of the distance from the common center. Thus, as the inner medulla is approached, a large decrease in the total cross-sectional area would indeed result, accounting for the increase in number densities of ascending limbs and collecting ducts.

For mathematical modeling of the transport processes, it would be of value to determine the total number of each tubule type at various medullary levels. Unfortunately, this is not possible since, as noted above, the areas of the medullary cross-sectional surfaces are in general not measurable. The area of the boundary between the cortex and medulla, however, was estimated in the contralateral kidney (Table 3). Since there are no cortical loops of Henle in the rat or rabbit [4], the total number of nephrons per kidney can be estimated as the product of the cortico-medullary surface area and the number density of ascending limbs at the cortico-medullary junction. When this calculation is done for rat R-4 (the only rat for which both numbers are available), a value of 37,500 is obtained. This agrees closely with estimates of 37,905 by Baines and DeRouffignac [26] and 38,029 by Barratt et al [27] for the total number of glomeruli per rat kidney, but is somewhat larger than the estimate of Rytand (30,800) [28]. Similarly, a value of 230,000 nephrons per rabbit kidney is estimated in rabbit H-2. This is only slightly greater than the value quoted by Rytand [28] for the total number of glomeruli in the rabbit kidney (207,000).

Another anatomical parameter of value in detailed analysis of renal transport is the number of initial collecting tubules which combine to form a single cortical collecting duct. This parameter can be estimated by the ratio of the number of ascending limbs to the number of collecting ducts at the cortico-medullary junction. The values obtained in the rats studied ranged from 5 to 6 (Fig. 7). This can be compared with Sperber's estimate of 5 [4]. The corresponding value in the rabbit is about 5 (Fig. 8), but to our knowledge, no literature value is available for comparison.

An important aim of this study has been to provide data which will allow more complete interpretation of physiologic transport data from the rat and the rabbit. The quantity of material transported by the nephron depends both on the properties of the membranes involved and the configuration of the transporting structures. Consequently, good anatomic data are as vital to the understanding of renal transport as is a knowledge of membrane transport properties. Full integration of anatomic data from this study with physiologic data will necessarily involve mathematical modeling and digital computer simulation [e.g., 25, 29] because of the complexity of the kidney and the extensive amount of data which must be incorporated into the analysis. Such techniques promise to lead to a much clearer understanding of the overall function of the kidney.

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